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(54) Composition for determination of human β_2-microglobulin, process for its preparation and its use

(57) A composition for use in the qualitative or quantitative determination of human β_2-microglobulin comprises synthetic polymeric latex particles having an anti-human β_2-microglobulin antibody coated on the surface of the latex particles. This composition may be prepared by contacting a synthetic polymeric latex having a solids concentration of from 0.05 to 5% with an anti-human β_2-microglobulin antibody having a concentration of 0.0001 to 1% in a buffer at a pH of from 5 to 10 at a temperature of from 4 to 40°C. The composition can be used to determine human β_2-microglobulins in human body fluids by, for example, the microtiter method.

GB2 030 294 A

SPECIFICATION

Composition for determination of human B_2-microglobulin, process for its preparation and its use

This invention relates to a composition for determining human β_2-microglobulin, a process for its production, and to its use. Specifically, it relates to a composition for determining human β_2-microglobulin (both qualitatively and quantitatively) which has superior storage stability and which can rapidly give reliable results with good reproductibility and a high degree of accuracy without being hampered by other proteins that may be contained in an assay sample, by using an easy measuring operation and a simple measuring instrument.

More specifically, this invention relates to a composition for determining human β_2-microglobulin, which is composed of particles of s synthetic polymeric latex, preferably particles of a styrene-type synthetic polymeric latex selected from the group consisting of styrene polymer, styrene copolymers and the carboxylated products of amino-containing carboxylated products thereof; to a process for preparation of the composition; and to use of the composition.

Human β_2-microglobulin is a protein having a relatively low molecular weight of about 12,000, and is found in the serum, urine, cerebrospinal fluid, saliva, milk, etc. of humans. The function *in vivo* of the β_2-microglobulin has not yet been fully elucidated. But it has recently been found that in certain diseases, such as inflammatory diseases, cancer, and renal diseases, the content of β_2-microglobulin in the patient's body fluids increases. For example, renal diseases are classisfied into those caused by glomerular disorder and those caused by disorder of renal tubules. In the latter type, the amount of β_2-microglobulin increases first in the blood, or more accurately, the serum, and then in the urine.

The β_2-microglobulin is a protein which permeates the glomerular basement membrane, and is then re-absorbed by the renal tubules. However,, when disorder of the renal tubules occurs, protein leaks into the urine without being reabsorbed. Accordingly, the level of β_2-microglobulin in the urine is one of the best parameters for the function of the renal tubules. Thus, disorder of the renal tubules can be diagnosed by determining the level of β_2-microglobulin in the serum and urine.

No suitable means of judging disorder of the renal tubules has been available in the past. However, it can now be easily diagnosed by measuring the β_2-microglobulin level in the serum and urine, thus opening a way for a suitable treatment of the disorder. This is very significant and useful for clinical application.

Development of a method has been desired which can determine human β_2-microglobulin in the serum or urine with a high level of accuracy and reliability. However, none of the conventional means for the determination of human β_2-microglobulin have proved to be satisfactory. One prior means is a single radial immunodiffusion method which comprises providing wells in an agar plate containing a human β_2-microglobulin antibody, filling an assay sample therein, and determining human β_2-microglobulin from a precipitation band which occurs with the diffusion of the β_2-microglobulin. Since this method has low sensitivity, it can only determine β_2-microglobulin in the assay sample in a concentration above a certain limit.

A radioimmunoassay method is also one of conventional means, which measures β_2-microglobulin by using a human β_2-microglobulin antibody labelled with a radioisotope. This method is dangerous and troublesome, however, since it deals with the radioactive substance. Accordingly, it cannot be performed unless sufficient control and equipment are available for using the radioactive substance.

Another example is a method using a reverse passive hemagglutination reaction (RPHA) which can easily determine β_2-microglobulin with an equivalent sensitivity to the radioimmunoassay. Since red blood cells of an animal are used as a carrier, this method has the disadvantage that in the preparation of antibody-sensitized red blood cells, pretreatments of the red blood cells, such as the fixation of red blood cells and treatment of the red blood cells with binding reagents such as glutaraldehyde or tannic acid are necessary. Furthermore, because a non-specific reaction attributed to an antibody for the red blood cells used as a carrier of ten occurs in actual measurement, it is necessary to perform an absorption treatment with red blood cells and a control experiment using tanned cells.

The present inventors have made investigations in order to provide a means for determining human β_2-microglobulin which is free from the defects and disadvantages of the conventional methods described hereinabove, and which can give reliable results with a high level of accuracy by using a simple instrument and a simple measuring operation. These investigations have led to the discovery that a composition comprising particles of a synthetic polymeric latex, preferably particles of a styrene-type synthetic polymeric latex, and a human β_2-microglobulin antibody coated on the surface of the particles has superior storage stability, and is very useful in determining the level of human β_2-microglobulin in an assay sample rapidly with a high level of accuracy and reliability and good reproducibility by using a simple measuring instrument and an easy measuring operation without being hampered by other proteins that may be contained in the assay sample. It has also been found that the aforesaid composition can be produced by an easy means, and can be stored not only as a latex but also as a dried product.

It is an object of this invention therefore to provide a novel and excellent composition for determining human β_2-microglobulin, a process for preparation thereof, and use of the aforesaid composition.

The above and other objects and advantages of this invention will become more apparent from the following description.

Human β_2-microglobulin is present in various human body fluids. Thus, the urine, milk, serum, etc. can be utilized as a starting human β_2-microglobulin for the preparation of the human β_2-microglobulin antibody in the composition of this invention. Most suitably, the starting material is prepared from the urine of a patient affected with the diseases mentioned hereinbove. Separation of the human β_2-microglobulin can be effected by utilizing various methods known *per se*. Examples of such methods include a zone electrophoretic method, the method of Berggård et al. (I. Bergård et al.: J. Biol. Chem., *243*, 4095, 1968) by ion-exchange chromatography, gel filtration, and the method of Ohsawa et al. (M. Ohsawa et al.: Experientia. 29, 1179, 1973).

The starting human β_2-microglobulin is desirably a single protein sample because the inclusion of impurities will complicate the subsequent operation.

An antibody of the human β_2-microglobulin that can be prepared by administering the β_2-microglobulin as an antigen to an animal having the ability to produce an antibody, such as guinea pigs, rabbits or goats, in a customary manner to immunize it, taking the blood from the immunized animal, and separating the human β_2-microglobulin antibody from the resulting blood.

The animal used in this method may be any animal which has the ability to produce antibodies. However, to obtain a large quantity of an antibody, the use of a large animal is preferred. Usually, rabbits or goats are suitable, but the animal is not limited thereto. Separation of a human β_2-microglobulin antibody from the antiserum containing it can be perormed by utilizing known means. For example, the antiserum is salted out, and adsorption-elution using an insoluble antigen is repeated.

Various synthetic latex particles can be used for the production of the composition for determining human β_2-microglobulin of this invention. Examples of polymers or copolymers that form such latex include, polystyrene, carboxylated polystyrene, amino-containing carboxylated polystyrene, polyvinyltoluene, styrene-butadiene copolymer, carboxylated styrene-butadiene copolymer, styrene-divinylbenzene copolymer, vinyl toluene-tertiary butyl styrene copolymer, polyesters, polyacrylic acid, polymethacrylic acid, polyacrylonitrile, acrylonitrile-butadiene-styrene copolymer, polyvinyl acetate acrylate, polyvinylpyrrolidone, and vinyl chloride-acrylate copolymer.

Preferred particles of synthetic polymeric latices are particles of styrene-type synthetic polymeric latices selected from the group consisting of styrene polymer, styrene copolymers such as a copolymer of styrene with a monomer selected from the group consisting of chlorostyrene, methyl methacrylate and vinylidene chloride, and carboxylated or amino-containing carboxylated products thereof. These synthetic polymerix latex particles can be used after pre-treating their surfaces with a non-ionic surface-active agent. For example, it is preferred to use these particles after causing an ethylene oxide-type nonionic surfactant to be adsorbed thereto in accordance with the method described in Japanese Laid-Open Patent Publication No. 9716/76 laid open on January 26, 1976. Examples of suitable nonionic surfactants of the ethylene oxide type are a block copolymer of ethylene oxide and polyoxypropylene glycol, polyoxyethylene alkyl ethers, and polyoxyethylene alkylaryl ethers.

In the present invention, the synthetic polymer latex particles preferably have an average particle diameter of 0.01 to 10 microns, more preferably 0.1 to 1 micron. To increase the reproducibility of the results of measurement, it is preferred to use particles having a relatively narrow range of size distribution. The specific gravity of the latex particles is preferably about 0.9 to 1.4, and more preferably about 1.1 to 1.3. In the case of measurement utilizing an agglutination reaction on a slide glass plate, latex particles having a broad range of specific gravity can be used. In the case of a microtiter method, it is preferable to use latex particles having a specific gravity of at least 1.1.

The human β_2-microglobulin determining composition of this invention can be produced by a simple means. For example, it can be produced by contacting a synthetic polymeric latex preferably having a concentration of 0.05 to 5% with a human β_2-microglobulin antibody having a concentration of 0.0001 to 1% in a buffer at a pH range of preferably about 5 to about 10, more preferably about 6.5 to about 8, at a temperature of preferably about 4 to about 40°C. The contacting can be performed with gentle stirring for about 30 minutes to about 24 hours.

The buffer used may, for example, be a phosphate-buffered saline [M/60 phosphate buffer (pH 7.2) containing 0.15M NaCl, to be abbreviated PBS], and a glycine-buffered saline. If desired, 0.01 to 0.1% of a protein such as bovine serum albumin (to be abbreviated BSA) may preferably be added to an antibody solution in order to prevent non-specific agglutination. After the coating reaction, the reaction mixture is washed several times by centrifugation in a neutral salt solution such as a glycine-buffered saline or PBS. Finally, the latex particles having the human β_2-microglobulin antibody coated thereon can be stored in the form of a suspension in a diluent. The diluent is preferably a mixture obtained by adding about 0.1% of BSA to a glycine-buffered saline or a PBS. It is further preferred to add 0.01 to 0.5% of sodium azide (naN_3) to the diluent.

The human β_2-microglobulin determining composition of this invention may be in the form of a latex as described above, and also in the form of a dried product. The composition in the form of a dried product can be obtained by adding a stabilizer such as an amino acid (e.g., glycine, or sodium glutamate) or dextran to the diluent described hereinabove, suspending the latex composition having human β_2-microglobulin antibody coated thereon in the diluent, and lyophilizing the suspension. The

amounts of the amino acid and dextran are about 1.2 to about 4 parts by weight, and about 1.6 to about 6 parts by weight, respectively per part by weight of the latex particles.

The human β_2-microglobulin determining composition of this invention has good stability both in the form of a latex and in the form of a dried product. The dried product can be stored stably for as long as several years.

According to this invention, there is provided a process for determining human β_2-microglobulin, which comprises qualitatively or quantitatively determining human β_2-microglobulin in the human urine or serum using a composition comprising synthetic polymer latex particles and a human β_2-microglobulin antibody coated on the surface of the latex particles.

For the determination, means known *per se* can be utilized, and for example, the level of β_2-microglobulin in the serum or urine can be easily determined by a microtiter method. According to this means, a certain amount of a diluent of the type exemplified hereinabove is poured portionwise onto a microplate, and then a certain amount of an assay sample such as the serum or urine is introduced into the first wall of the plate and successively diluted with a diluter. On the other hand, a dilution series is prepared in the same way using a standard antigen. A certain amount of a human β_2-microglobulin antibody-sensitized latex is added to each of them and mixed. After standing for a certain period of time at room temperature, the end point of aggultination is observed, and the absolute amount of β_2-microglobulin can be determined by comparison with the standard antigen series.

In the conventional single radial immunodiffusion method for determination of β_2-microglobulin, the β_2-microglobulin cannot be determined unless its amount is at least 5 μg per ml of an assay sample when the assay sample is the serum or urine. Since an error is liable to occur with a β_2-microglobulin content of about 5 to 10 μg, a somewhat higher concentration is preferred. In contrast, according to the method for determining β_2-microglobulin in accordance with this invention using the human β_2-microglobulin antibody-sensitized latex, determination is possible if the amount of β_2-microglobulin is more than 1 ng per ml. Thus, this method shows a very high sensitivity, and the sensitivity is equivalent to, or higher than, that of radioimmunoassay which is considered to have the highest sensitivity in ordinary analyses.

According to another embodiment of measurement in accordance with this invention, a certain amount of an assay sample is dropped to a slide glass plate, and separately, a fixed amount of a human β_2-microglobulin solution of a known concentration is added dropwise. A certain amount of the human β_2-microglobulin antibody-sensitized latex is added to each of them, followed by gentle oscillation. By evaluating the resulting agglutination pattern, the concentration of the human β_2-microglobulin in the assay sample can be qualitatively determined within a few minutes. Semi-quantitative determination can also be carried out if the assay sample is diluted in a test tube and the reaction is observed with respect to each dilution.

The human β_2-microglobulin measuring composition in the form of latex or dried product is superior to compositions obtained by coating human β_2-microglobulin on red blood cells or another carrier in that because of the lack of antigenicity of the carrier itself, non-specific reactions other than the desired antigen-antibody reaction do not take place; that a human β_2-microglobulin antibody can be coated directly on a latex easily by simply dipping an antibody solution in the latex without using a binder; and that it has good storage stability.

The novel composition of this invention comprising latex particles and a human β_2-microglobulin antibody coated thereon does not at all react with proteins other than the human β_2-microglobulin, and the carrier neither reacts with β_2-microglobulin. From this fact, it can be said that the human β_2-microglobulin antibody is bonded to the carrier latex particles.

The present invention is described in greater detail below by Preparation Examples and Working Examples. It should be understood that the invention is not limited by these examples so long as it does not depart from the scope and spirit thereof.

PREPARATION EXAMPLE 1

Preparation of human β_2-microglobulin:—

One kilogram of ammonium sulfate was added to about 2 litres of the urine of a patient affected with nephritis, and the solution was allowed to stand overnight at 4°C. The precipitate formed was collected, and dissolved in water. The insoluble matter was separated by centrifugation, and the precipitate was dissolved in saline and applied to a Sephadex G100 (a product of Pharmacia Fine Chemicals Co., Ltd., Sweden) column (5 x 100 cm) buffered with a 0.02M Tris buffer (pH 8.0) containing 1M sodium chloride. The buffer was allowed to flow at a flow rate of 40 ml per hour to perform gel filtration. 400 ml of eluted fractions were collected, desalted, and concentrated to a volume of 10 ml. Then, the product was applied to a DEAE cellulose (a product of Brown Company, U. S. A.) column (2.5 x 40 cm) buffered with 0.01M phosphate buffer (pH 7.5) and elution was performed while linearly increasing the concentration of sodium chloride in the buffer to 0.2M. 70 ml of a protein fraction which was eluted in the first place was collected, de-salted, and concentrated to 10 ml. The liquid was applied to an SP-Sephadex C 50 (Pharmacia Fine Chemicals Co., Ltd., Sweden) column (2 x 35 cm) buffered with a 0.04M phosphate buffer (pH 5.9), and elution was performed at a flow rate of 25 ml per hour while linearly increasing the concentration of the buffer to 0.2M. One hundred milliliters of

fractions from 75 ml to 175 ml of eluates were collected, de-salted, concentrated, and lyophilized to afford 60 mg of a white powder.

The product showed a single band in disc electrophoresis.

PREPARATION EXAMPLE 2

Preparation of human β_2-microglobulin antibody:—

The human β_2-microglobulin obtained by Preparation Example 1 was dissolved in a physiological saline in a concentration of 4 mg per ml of the latter. The solution was mixed with an equal amount of a complete Freunds adjuvant. The mixture was injected in an amount of 0.1 ml to each foot pad of four healthy rabbits weighing about 2.3 to 2.5 kg three times every week. On week later, 1 ml of a 0.1% human β_2-microglobulin saline was injected. Three weeks after the final injection, the whole blood was taken from the carotid artery of the rabbits. The blood was centrifuged in a customary manner, and the resulting serum was maintained at 56°C for 30 minutes to form about 200 ml of antiserum.

The remaining antiserum was determined to be a single antibody from the fact it formed a single precipitation band with an antigen by an Ouchtalony method and immunoelectrophoresis.

PREPARATION EXAMPLE 3

Preparation of insoluble human β_2-microglobulin:—

5 mg of human β_2-microglobulin was dissolved in 5 ml of a 0.2M acetic acid buffer (pH 5.0), and about 0.5 ml of a 5% bovine serum albumin solution was added. Then, 5% glutaraldehyde was added dropwise until a precipitate formed. The precipitate was collected, homogenized, and washed with PBS and then with a glycine-hydrochloric acid buffer (pH 2.8) and further with PBS, and stored at below −20°C.

PREPARATION EXAMPLE 4

Purification of human β_2-microglobulin antibody:—

To antiserum was added an equal amount of a saturated solution of ammonium sulfate, and they were fully mixed. The mixture was allowed to stand at room temperature for 30 minutes. The precipitate that formed was collected by centrifugal separation, washed with 0.5 saturated solution of ammonium sulfate, and then dialyzed against PBS. Then, the insoluble human β_2-microglobulin prepared in Preparation Example 3 was added to the dialyzate, and then the mixture was allowed to stand at room temperature for 30 minutes. The mixture was centrifuged to separated it into a supernatant liquid and a sediment. To the supernatant liquid was again added insoluble human β_2-microglobulin, and the mixture was similarly treated. The two precipitates were combined, and washed with PBS. Then, a glycine-hydrochloric acid buffer (pH 2.8) was added, and the mixture was shaken for 5 minutes, followed by centrifugation. The precipitate was similarly treated with a glycine-hydrochloric acid buffer, and combined with the resulting supernatant liquid. The mixture was dialyzed against PBS to form a purified human β_2-microglobulin antibody.

WORKING EXAMPLE 1

Human β_2-microglobulin antibody-sensitized latex:—

Polystyrene latex (a product of Takeda Chemical Industry Co., Ltd.; SDL 59; specific gravity 1.14; particle diameter 0.9 micron) was diluted with PBS so that the concentration of the particles became 0.25%, and then an equal amount of a purified antibody diluted to 60 times with PBS was added. The mixture was maintained at 37°C for 2 hours, and centrifuged. The latex particles were collected, and washed with PBS and then with a diluent. Then, the latex particles were suspended to a concentration of 0.25% using a diluent to form a human β_2-microglobulin antibody-sensitized latex.

When a human β_2-microglobulin solution was added to the sensitized latex, agglutination occurred. But no agglutination occurred when albumin, IgG, IgM and IgA solutions were respectively added to it.

The diluent used was PBS containing 0.08% BSA and 0.1% sodium azide.

WORKING EXAMPLE 2

Determination of human β_2-microglobulin:—

A diluent in an amount of 0.025 ml was poured into each well of a V-type microplate, and 0.025 ml of serum or serum diluted with a suitable diluent was added to the first well, and diluted successively with a diluter by a serial twofold dilution method. On the other hand 0.025 ml of a standard solution containing 0.01 μg of human β_2-microglobulin per ml was added to the first well of another row, and similarly diluted. Then, 0.025 ml of the human β_2-microglobulin antibody-sensitized latex was poured in each of the wells, and after sufficient mixing, was allowed to stand at room temperature for more than 10 hours to observe the end point of agglutination. When an antigen-antibody reaction occurred, the latex particles were dispersed on the entire surface of the bottom of a well. When no antigen-antibody reaction occurred, the latex particles gathered at the center of each well. Thus, the end point of agglutination can be judged.

In accordance with the above procedure, the amount of β_2-microglobulin was determined with regard to seven examples of serum diluted to 100 times and seven examples of urine diluted to 50 times. The results are shown in Table 1.

TABLE 1: Latex agglutination value

Sample \ No.		1	2	3	4	5	6	7	8	Concentration in the sample (ng/ml)
Concentration of standard β_2-microglobulin (ng/ml)		5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	
Agglutination pattern		+	+	+	+	−	−	−	−	
Serum	Healthy adult (32 years old male)	+	+	+	+	−	−	−	−	1
	Healthy adult (43 years old male)	+	+	+	−	−	−	−	−	0.5
	Healthy adult (22 years old male)	+	+	+	+	−	−	−	−	1
	Leukemic patient (9 years old male)	+	+	+	+	+	+	+	−	8
	53 years old male affected with cancer at the stomach	+	+	+	+	+	+	+	−	8
	62 years old male affected with renal failure	+	+	+	+	+	+	+	+	above 16
	32 years old female affected with SLE	+	+	+	+	+	+	+	−	8
Urine	Healthy adult (32 years old male)	+	+	+	−	−	−	−	−	0.25
	Healthy adult (43 years old male)	+	+	−	−	−	−	−	−	0.13
	Healthy adult (22 years old male)	+	+	+	−	−	−	−	−	0.25
	57 years old male affected with myeloma	+	+	+	+	+	+	+	−	4
	9 years old male affected with leukemia	+	+	+	+	+	+	+	−	4
	62 years old male affected with renal failure	+	+	+	+	+	+	+	+	above 8
	32 years old female affected with SLE	+	+	+	+	+	+	+	−	4

+: positive; −: negative.

WORKING EXAMPLE 3

Determination of human β_2-microglobulin by slide aggregation:—

Polystyrene latex (a product of Dow Chemical; particle diameter 0.22 ± 0.006 micron; specific gravity 1.05) was diluted to 1% with a glycine-sodium chloride buffer (pH 8.4), and an equal amount of a purified antibody was added. The mixture was allowed to stand overnight at 4°C, and then centrifuged to separate the latex. The latex was washed once with a glycine-sodium chloride buffer, and suspended to a concentration of 1% in a glycine-sodium chloride buffer containing 1% BSA to form a sensitized latex.

50 μl of a human β_2-microglobulin solution having a concentration of 400 ng/ml, 200 ng/ml, 100 ng/ml, 50 ng/ml, or 25 ng/ml was added dropwise separately on a slide glass. Then, 50 μl of the resulting sensitized latex was added dropwise on top of it, and with gentle stirring for a few minutes, the reaction was observed. An agglutination pattern appeared when the concentration of the human β_2-microglobulin solution was 400 ng/ml, 200 ng/ml, and 100 ng/ml, but no agglutination pattern appeared at a concentration of 50 ng/ml and 25 ng/ml.

By the above procedure, the content of human β_2-microglobulin was estimated with regard to 6 examples of serum and 6 examples of urine. The results are shown in Table 2.

TABLE 2 : Latex agglutination value

Assay Sample	Agglutination pattern	Concentration in the sample
Serum (diluted to 50 times)		
Healthy adult (32 years old male)	–	less than 2.5 g/ml
Healthy adult (43 years old male)	–	,,
Healthy adult (22 years old male)	–	,,
Leukemic patient (9 years old male)	+	at least 5 g/ml
62 years old male affected with renal failure	+	,,
32 years old female affected with SLE	+	,,
Urine (diluted to 10 times)		
Healthy adult (32 years old male)	–	less than 0.5 g/ml
Healthy adult (43 years old male)	–	,,
Healthy adult (22 years old male)	–	,,
Leukemic patient (9 years old male)	+	at least 1 g/ml
62 years old male affected with renal failure	+	,,
32 years old female affected with SLE	+	,,

Control					
Concentration of standard β_2-microglobulin (ng/ml)	400	200	100	50	25
Agglutination pattern	++	+	+	±	–

WORKING EXAMPLE 4

By treating in the same way as in Working Example 1, a latex was sensitized, washed, and suspended in a diluent containing 0.5% of glycine and 0.7% of Dextran T—10 (a product of Pharmacia Fine Chemicals Co., Ltd., Sweden) so that the concentratoin of the latex particles became 1.25%. The suspension was then lyophilized in a customary manner to obtain a composition of latex particles sensitive to a human β_2-microglobulin antibody.

WORKING EXAMPLE 5

A diluent was added to the latex particle composition sensitive to human β_2-microglobulin antibody obtained in Working Example 4. Then, by operating in the same way as in Working Example 2, the amount of β_2-microglobulin in the serum and urine was determined. Similar results were obtained.

As is clearly seen from the results obtained in the above Examples, the amount of β_2-microglobulin in the serum and urine of patients affected with various diseases showed an apparently higher value than that of the sera and urines of healthy adults. Accordingly, the determination of the amount of β_2-microglobulin in the blood or urine is very useful for diagnosis of these diseases.

Separately, the amount of β_2-microglobulin in the serum and urine of humans was measured by using a human β_2-microglobulin antibody-sensitized latex prepared from the antiserum of a goat in the same way as in Preparation Example 4 and subsequent Working Examples. The results were quite the same as in the above tables.

By using the human β_2-microglobulin antibody-sensitized latex or the composition containing the sensitized latex in accordance with this invention, the amount of β_2-microglobulin can be measured within a shorter period of time if there is a sample (human serum or urine) in a very small amount of less than 0.03 ml. The sensitivity is very high, and 1 ng of β_2-microglobulin can be measured per ml of the assay sample. Accordingly, the composition of this invention affords significant and useful information for the diagnosis of leukemia, renal diseases, inflammatory diseases, etc., and its range of application is broad. Furthermore, since the amount of the assay sample can be very small, it is especially suitable for children, especially neonates and infants, from whom only a small amount of blood can be taken.

CLAIMS

1. A composition for use in the qualitative or quantitative determination of human β_2-microglobulin comprising synthetic polymeric latex particles having an anti-human β_2-microglobulin antibody coated on the surface of the latex particles.

2. A composition according to claim 1 wherein the latex particles have an average particle diameter of from 0.01 to 10 microns.

3. A composition according to claim 1 or 2 wherein the latex particles have a specific gravity of from 0.9 to 1.4.

4. A composition according to any one of the preceding claims which further comprises a stabilizer which is an amino acid, a salt thereof, or dextran.

5. A composition according to any one of the preceding claims wherein the synthetic polymeric latex particles are particles of a latex of a styrene homopolymer, a styrene copolymer or carboxylated or amino-containing carboxylated products thereof.

6. A composition according to any one of the preceding claims which is in the form of a latex.

7. A composition according to any one of the preceding claims which is in the form of a dried product.

8. A composition according to claim 1 substantially as hereinbefore described with reference to any one of Working Examples 1, 3 or 4.

9. A process for producing a composition as claimed in any one of the preceding claims, which process comprises contacting a synthetic polymeric latex having a solids concentration of from 0.05 to 5% with an anti-human β_2-microglobulin antibody having a concentration of 0.0001 to 1% in a buffer at a pH of from 5 to 10 at a temperature of from 4 to 40°C.

10. A process according to claim 9 substantially as hereinbefore described with reference to any one of Working Examples 1, 3 or 4.

11. A method for the qualitative or quantitative determination of human β_2-microglobulin, which method comprises causing any human β_2-microglobulin contained in a sample of a human body fluid to be brought into contact with a composition as claimed in any one of claims 1 to 8 or which has been produced by a process as claimed in claim 9 or 10.

12. A process according to claim 11 wherein the human fluid is urine or serum.

13. A method according to claim 11 or 12 wherein the determination is carried out by a microtiter method.

14. A method according to claim 11 substantially as hereinbefore described with reference to any one of Working Examples 2, 3 or 5.

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